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27.03.91 Bulletin 91/13(71) Applicant: **ORION-YHTYMÄ OY**
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W-8000 München 86(DE)(54) **Method and reagent combination for determining nucleotide sequences.**

(57) The invention provides for detection of changes in a nucleotide sequence based on the determination of the nucleotide sequence over the region of interest. The first step of the process involves obtaining an amplified DNA sample in which at least one attachment moiety has been introduced into at least one strand of a target polynucleotide by a modified polymerase chain reaction. This can be accomplished by synthesizing DNA using the target nucleic acid polymer as a template and using at least one amplification primer, which includes an attachment moiety bonded to the oligonucleotide. The resulting amplification mixture is repeatedly cycled between a polymerization condition and a denaturation condition occurs for a number of cycles sufficient to produce copies of the target nucleic acid polymer in a quantity suitable for use in a DNA sequencing procedure to form an amplified sample comprising copies of the target nucleic acid sequence bonded to the attachment moiety. After amplification, the strands of target nucleic acid polymer carrying an attachment moiety are captured using a solid matrix coated with an attachment site to which the attachment moiety or a modification thereof can bind. In this way, the strands of target nucleic acid become bound to the solid matrix and can be readily separated with the solid matrix from unbound reactants for sequencing.

rated with the solid matrix from unbound reactants for sequencing.

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EUROPEAN SEARCH REPORT

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	GB-A-2 202 328 (ORION-YHTYMA OY) * Whole document *	1-5, 13-21,25, 30-32,34	C 12 Q 1/68 G 01 N 33/53 // C 07 H 21/04
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES USA, vol. 85, January 1988, pages 544-548; D.R. ENGELKE et al.: "Direct sequencing of enzymatically amplified human genomic DNA" * Pages 544-545 *	1-5, 13-21,25, 30-32,34	
P,X	NATURE, vol. 330, 26th November 1987, pages 384-386; C. WONG et al.: "Characterization of beta-thalassaemia mutations using direct genomic sequencing of amplified single copy DNA" * Whole document *	1-5, 13-21,25, 30-32,34	
P,X	WO-A-8 909 282 (M.J. HOLMES) * Abstract; claims 7,8,10-12; page 7, line 5 - page 8, line 28; page 13, paragraph 4; pages 16-20 *	1-5,13-15	
P,X	MEETING ON THE POLYMERASE CHAIN REACTION: METHODOLOGY AND APPLICATIONS, 3rd - 7th April 1989; & J. CELL. BIOCHEM., suppl. 0, (13 part E), 1989, page 298, New York, US; L.G. MITCHELL et al.: "Affinity generation of single stranded DNA following the polymerase chain reaction: application to dideoxy sequencing"	1-5	TECHNICAL FIELDS SEARCHED (Int. Cl.5) C 12 Q
E	WO-A-8 912 063 (THE UNITED STATES OF AMERICA) * Abstract; claims *	1-5	
A	WO-A-8 605 815 (GENETICS INTERNATIONAL INC.) * Abstract; claims *	11	
The present search report has been drawn up for all claims			
Place of search The Hague		Date of completion of search 14 December 90	Examiner MOLINA GALAN E.
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention</p> <p>E: earlier patent document, but published on, or after the filing date O: document cited in the application L: document cited for other reasons &: member of the same patent family, corresponding document</p>			



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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	<p>NUCLEIC ACIDS RESEARCH, vol. 16, no. 7, 1988, pages 3025-3028, IRL Press Ltd, Oxford, GB; S. STAHL et al.: "Solid phase DNA sequencing using the biotin-avidin system"</p> <p>-----</p>		
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
The present search report has been drawn up for all claims			
Place of search		Date of completion of search	Examiner
The Hague		14 December 90	MOLINA GALAN E.
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention</p> <p>E: earlier patent document, but published on, or after the filing date D: document cited in the application L: document cited for other reasons</p> <p>&: member of the same patent family, corresponding document</p>			